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Commentary

Reproductive and developmental toxicity testing: Examination of the extended one-generation reproductive toxicity study guideline

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ABSTRACT

An important aspect of safety assessment of chemicals (industrial and agricultural chemicals and pharmaceuticals) is determining their potential reproductive and developmental toxicity. A number of guidelines have outlined a series of separate reproductive and developmental toxicity studies from fertilization through adulthood and in some cases to second generation. The Extended One-Generation Reproductive Toxicity Study (EOGRTS) is the most recent and comprehensive guideline in this series. EOGRTS design makes toxicity testing progressive, comprehensive, and efficient by assessing key endpoints across multiple life-stages at relevant doses using a minimum number of animals, combining studies/evaluations and proposing tiered-testing approaches based on outcomes. EOGRTS determines toxicity during preconception, development of embryo/fetus and newborn, adolescence, and adults, with specific emphasis on the nervous, immunological, and endocrine systems, EOGRTS also assesses maternal and paternal toxicity. However, EOGRTS guideline is complex, criteria for selecting doses is unclear, and monitoring systemic dose during the course of the study for better interpretation and human relevance is not clear. This paper discusses potential simplification of EOGRTS, suggests procedures for relevant dose selection and monitors systemic dose at multiple life-stages for better interpretation of data and human relevance.

1. Background

In order to provide a background for discussion of the EOGRTS guidance, the readers should be aware of several other guideline studies routinely conducted, primarily in rats, to determine immediate and latent reproductive effects of chemical exposure. Assessment of toxicity to reproduction includes possible effects of chemicals on fertility, embryonic and fetal development, peri- and postnatal development, and maternal function. Traditionally, separate reproductive/developmental toxicity studies are conducted to evaluate these effects. Guidelines OECD 414 and OPPTS 870.3700 determine effects of chemicals on embryo-fetal development/death, altered growth and structural changes (ICH, 2005; OECD, 2001a; USEPA, 1998a). Effects of chemicals on maternal behavior, length of gestation, dystocia, number and sex of pups, live births, runts, presence of gross abnormalities, and abnormal behavior in pups are determined in guidelines OECD 421 (screening test) and OPPTS 870.3550 (ICH, 2005; OECD, 1995; USEPA, 2000a). General and reproductive/developmental toxicity endpoints are combined in OECD 422 (screening test) and OPPTS 870.3650 guidelines (OECD, 1996; USEPA, 2000b).

Guidelines OECD 415 and 416 determine effects of chemicals on reproduction in one- and two-generation studies, respectively (OECD, 1983, 2001b; USEPA, 1998b). The two-generation study (OECD 416; OPPTS 870.3800) is considered the most comprehensive design to assess reproductive toxicity (Carney and Sattivari, 2013) and the effects of chemicals on the reproductive performance of the F1 parents. The two-generation study assesses effects of chemicals on reproductive parameters listed for OECD 421 in P and F1 generations as well as the presence of gross abnormalities and abnormal behavior in F1 and F2 animals. The NTP's modified one-generation study design determines effects of chemicals on animals from gestation through weaning of F2 animals (Foster, 2014); however, no formal guideline document exists. The difference between the NTP design and other approved guidelines include retention of multiple pups per litter rather than 1 pup/sex/litter/dose group and premating treatment of males for a full 10

Abbreviations: EOGRTS, Extended One-Generation Reproductive Toxicity Study; DNT, Developmental Neurotoxicity; DIT, Developmental Immunotoxicity.

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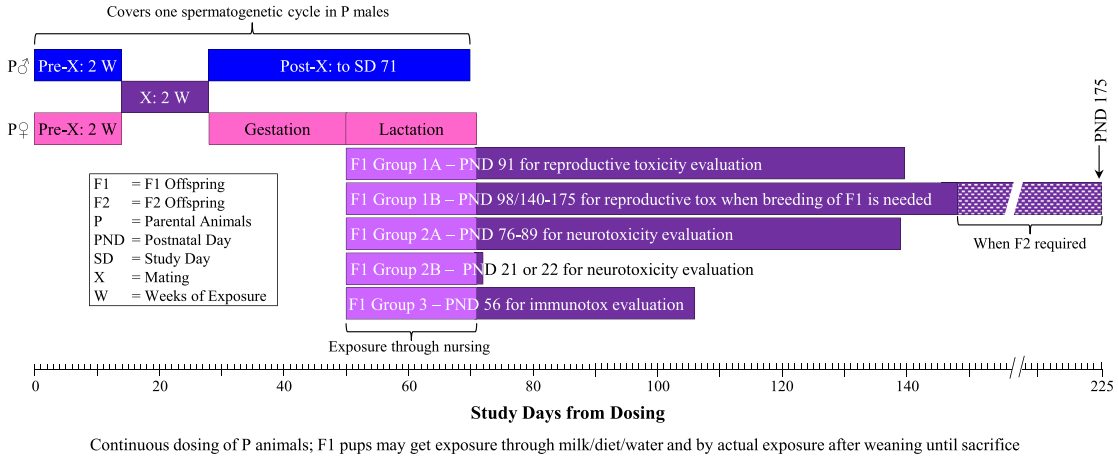


Fig. 1. Graphic depiction of the current EOGRTS (OECD 443) design.

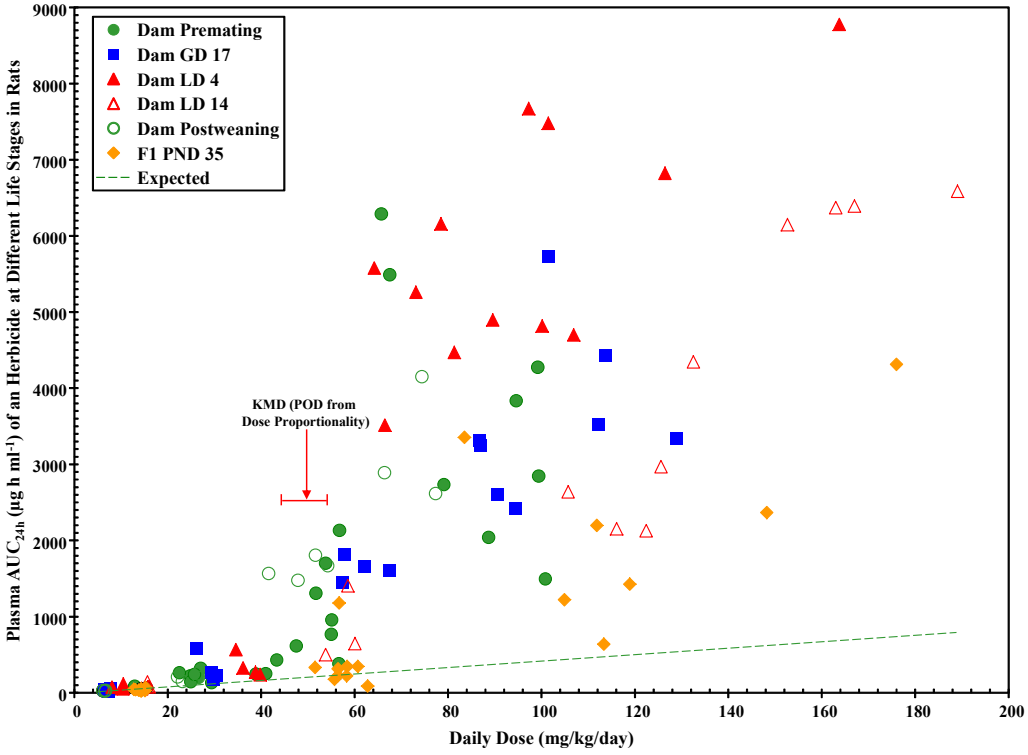


Fig. 2. Change in systemic dose of a herbicide (2,4-D) in rats at different life stages and determination of the kinetically-derived maximum dose (modified from Saghir et al., 2013).

weeks. However, both ICH and OECD guidelines indicate that a full 10-week premating period is often not needed, especially when other general toxicity studies (e.g., existing subchronic studies) indicate a lack of toxicity to the testes or uterus.

2. Current guidelines and modified approach

Most of the above described individual guidelines evaluate toxicity of chemicals to only parts of the reproductive and developmental stages with the exception the two-generation reproductive toxicity study. These guideline studies have not been updated to reflect advancements in the assessment of developmental and reproductive toxicity. For example, researchers now like to combine multiple reproductive and developmental toxicity studies into a single study and determine systemic exposure during dose range-finding or other general toxicity studies for the selection of appropriate doses (Chapman et al., 2013; Dorato et al., 2014; Marty et al., 2013; Saghir et al., 2013). Although the two-generation toxicity study is considered “the gold standard” for the assessment of reproductive toxicity, it is complex in design, high in the utilization in animals (~2600 animals for study in rats) and with debatable value of the F2 generation (Janer et al., 2007a, 2007b; Moore et al., 2009; Piersma et al., 2011; Rorije et al., 2011). The two-generation toxicity study is also not designed to evaluate developmental neurotoxicity (DNT) or developmental immunotoxicity (DIT) endpoints, which require standalone studies using an additional 1280 animals.

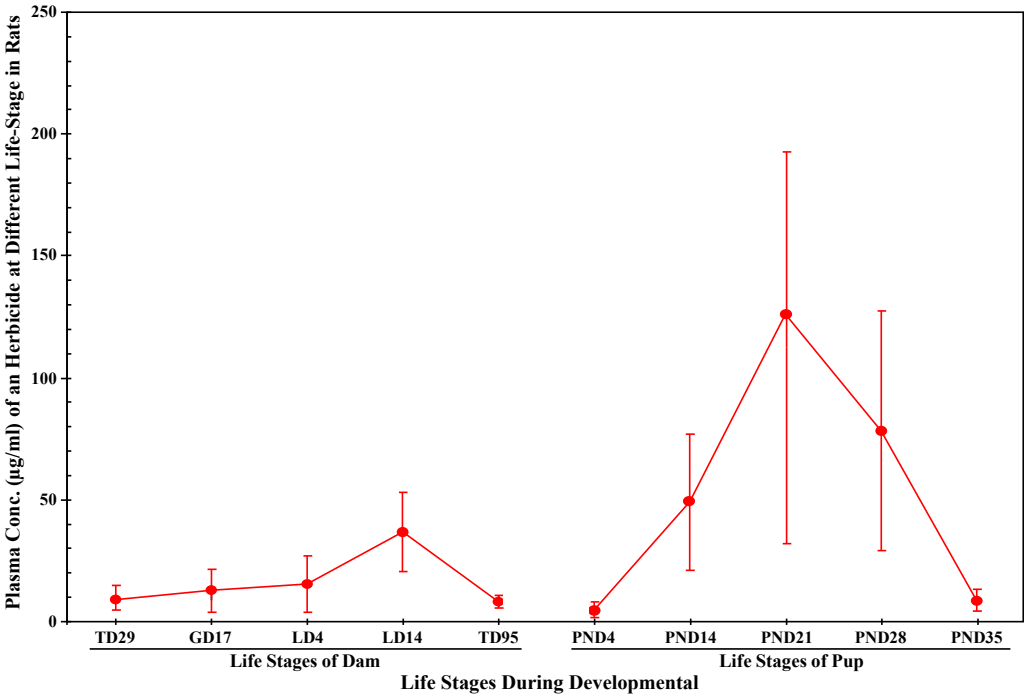
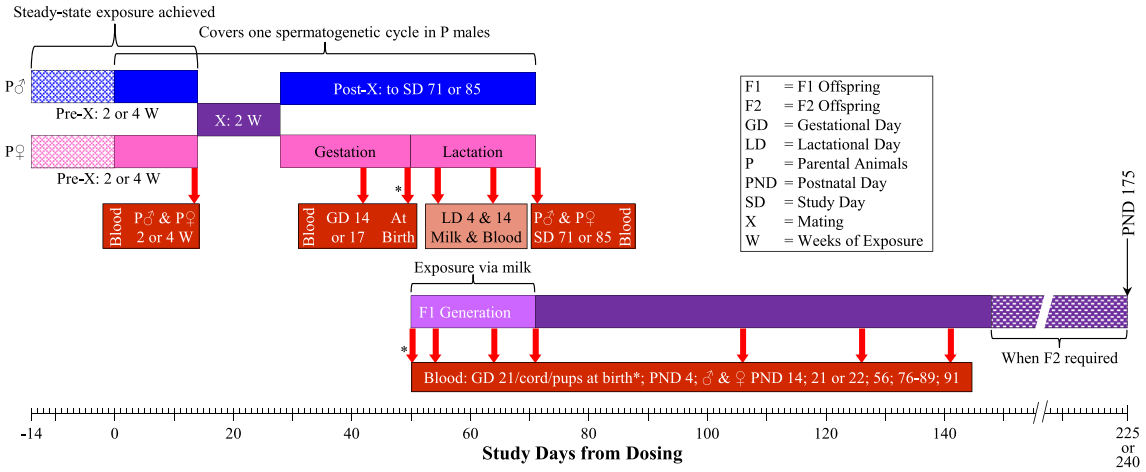


Fig. 3. Change in systemic dose of a herbicide (2,4-D) at different life stages of rats (modified from Saghir et al., 2013).



Continuous dosing of P animals; F1 pups may get exposure through milk/diet/water and by actual exposure after weaning until sacrifice.
Blood and/or milk sampling days may vary slightly depending upon the fate of the chemical in rats and pertaining issues (see Tables 1-4 and text for detail).
Two additional weeks of dosing of P animals may be required depending upon the kinetic behavior of a chemical in rats.
*Blood may be obtained from dams during gestation and from dams and pups (or cord blood) at birth or even from dams and fetuses one day prior to parturition from animals used in the dose-range-finding study or from dedicated groups in the main study (see Tables 1-4 and text for detail).

Fig. 4. Graphic depiction of the improvements to EOGRTS (OECD 443) design with scheme for the determination of systemic dose at different life-stage (reproductive/developmental landmarks) during study using core study animals.

2.1. Extended one-generation reproductive toxicity study (EOGRTS)

To make the toxicity testing across life stages state-of-the-art, the International Life Sciences Institute/Health and Environmental Science Institute (ILSI/HESI) Agricultural Chemicals Safety Assessment (ACSA) Technical Committee was charged with proposing an improved testing paradigm to assess potential effects of chemicals across life stages by incorporating the current understanding of developmental and reproductive toxicity (ILSI/HESI, 2001; Cooper et al., 2006). The committee identified key toxicity profiles across life stages beyond developmental and reproductive phases, combined studies/evaluations of endpoints across multiple life stages, and proposed a tiered testing approach for flexibility based on the needs and available data. The committee considered approaches to assess the potential of chemicals to cause adverse effects on reproduction, developmental life stages, and in the elderly. The life stage toxicity was defined as the potential adverse effects of chemicals on preconception, development (embryo/fetal and newborn/pre-weaning life stages), adolescence, and adults of all ages for reproductive and developmental toxicity, any special sensitivity with respect to general toxicity and specific effects on the nervous, immunological, and endocrine systems at critical life

Table 1
Outline of EOGRTS for parents including systemic dose determination.

Prior to and during cohabitation	
Housing prior to cohabitation	Group by sex
Housing during cohabitation	1:1 male:female
Clinical observations (CO)	≥ twice daily
PE	SD1 and weekly
BW	SD1 and weekly
FC	Weekly
Vaginal smear	Daily for 2 weeks
Blood[a]	***SD 14 or 28, test chemical concentration at SS (2 or 4 weeks)***
Males following cohabitation[b]	
Housing	Group
CO, PE, BW, FC	Same as above
Blood[c]	***At sacrifice, SD 71 or 85, test chemical concentration***
Sperm analysis	At sacrifice, SD 71, using one testis/epididymis/vas deferens
Urine	Urinalysis
Blood	Hematology, Clinical Path, T4, TSH
Tissues	Histopathology
During gestation	
Housing	Dam
CO, PE, FC	Same as above
BW	Every 2 days
Blood[d]	***GD 14 or 17, test chemical concentration***
At birth	
Blood[e]	***Dam for test chemical concentration***
During lactation	
Housing	Dam and pups
CO, PE, FC	Same as above
BW	LD 4, 7, 14, 21
Blood[d]	***LD 4 and 14 for test chemical concentration***
Milk[f]	***LD 4 and 14 for test chemical concentration***
At termination[b]	
Urine[g]	Urinalysis
Blood[d,g]	Hematology, Clinical Path, T4, TSH, ***test chemical concentration***
Tissues	Histopathology
Uteri	All: presence and number of implantation site
Vaginal smears	All: stage of estrous cycle for correlation with histopathology

BW, body weight; CO, clinical observation; FC, food consumption; GD, gestational day; LD, lactational day; PE, physical examination; SD, study day; SS, steady-state; T4, thyroxin; TSH, thyroid stimulating hormone.
Twenty-four hour or spot urine samples may be collected on days of blood collection to determine test chemical concentration.
Bold italic texts are proposed additions to improve the current EOGRTS design.
[a] Randomly selected 4 ♂ and 4 ♀ for systemic dose determination (one ≤100 μl blood, see text for time).
[b] As outlined in the protocol for hematology, clinical biochemistry, T4, TSH, urinalysis, gross pathology and tissues for histopathology.
[c] Ideally same randomly-selected parental male rats for systemic dose determination (one ≤100 μl blood, see text for detail).
[d] Ideally same randomly selected dams for systemic dose determination (one ≤100 μl blood, see text for time).
[e] For placental transfer, collect ≤100 μl blood from dam and her pups (or placenta), see text for detail.
[f] For lactational transfer, collect ≤100 μl milk likely from extra or dose-range-finding animals (see text for detail).
[g] From randomly-selected 4–5 ♂ and ♀ per dose group.

stages. Additionally, they emphasized using doses that are relevant to realistic human exposures while maintaining adequate power to detect toxicity utilizing a systemic dose in a minimum number of animals (see Cooper et al., 2006; Marty et al., 2013; Saghir, 2015; Saghir et al., 2012, 2013).

The ILSI/HESI-ACSA proposed study design (Cooper et al., 2006) became the basis for the OECD 443 EOGRTS guideline (OECD, 2012). The study starts with exposing a sufficient number of adult male and female rats (to achieve 20 litters/dose) to the test chemical for two weeks prior to mating through weaning. Both parents are then sacrificed on study day (SD) 71 and evaluated while pups are continuously dosed with the test chemical until their scheduled sacrifice after evaluation for possible toxicological effects (Fig. 1). Groups of pups are evaluated for developmental neurotoxicity and at sexual maturity for reproductive, immuno, neuro, and general toxicity, and bred, when triggered, to produce F2 litters. The trigger to generate F2 animals in EOGRTS is based on developmental landmarks (e.g., anogenital distance, nipple retention, puberty onset) in F1 animals. In addition to the enhanced interpretative value, the EOGRTS protocol also retains multiple pups per litter, similar to the NTP study design and in contrast to retaining 1 pup/sex/dose in conventional two-generation reproductive toxicity study protocols (Marty et al., 2013). Therefore, it is not clear how the NTP design offers additional advantage as mentioned by Foster (2014). Feasibility/validation of EOGRTS was achieved in four studies conducted for 2,4- dichlorophenoxyacetic acid (2,4-D) methimazole, vinclozolin, and lead acetate (Fegert et al., 2012; Marty et al., 2013; Milius et al., 2010; Schneider et al., 2011; Wright et al., 2011).

Although, the EOGRTS approach provides advantage by combining evaluations, adding DNT and DIT parameters and decreasing animal use, it is not without criticism. Even though Schiffelers et al. (2015) raised concern about the acceptance of the current EOGRTS protocol in the Europe without amendments due to criticism, the European Commission has recently adopted the EOGRTS (EC, 2015). However, the Commission has left an option for European Chemicals Agency (ECHA) to request performance of the F2 generation when justified (EC, 2015). In addition to the debate on the limited added value of the second generation (Janer et al., 2007a, 2007b; Martin et al., 2009; Piersma et al., 2011; Rorije

Table 2
Outline of EOGRTS for F1 including systemic dose determination.

Blood samples[a]	***GD 21 or cord or pups at birth, selected animals for test chemical concentration***
At birth	Unique litter and group identification on PND 0 or 1
Culling	Reduce to 5 males and 5 females per litter on PND 4
Blood samples[b]	From culled/litter/group for T4, TSH ***and test chemical concentration***
Angiogenital Distance	Males PND 4
Gross necropsy	All culled pups on PND 4
Housing until weaning	Litters with respective mothers
Nipple assessment	Male pups PND 12 or 13
Housing after weaning	Small groups of same sex and treatment
Clinical observations	$\geq$ twice daily
Physical examination	Weekly at the time of weighing
BW before weaning	PND 4, 7, 14, 21 (at weaning)
BW after weaning	At weaning and weekly thereafter[c]
Food consumption	Weekly following assigning to cohorts
Blood samples[d]	PND 22 for T4, TSH ***and test chemical concentration***
Sacrifice	Gross necropsy of pups not selected for cohort on PND 22
Tissues[e]	Brain, spleen, thymus, mammary gland, target tissues on PND 22
Blood samples[f]	***PND 14, 56, between 76 and 89, 91, selected animals for test chemical concentration***
Maturity assessment[g]	Evaluated daily starting before the expected day in all selected animals

BW, body weight; PND, postnatal day; T4, thyroxin; TSH, thyroid stimulating hormones.
Twenty-four hour or spot urine samples may be collected on days of blood collection to determine test chemical concentration.
Bold italic texts are proposed additions to improve the current EOGRTS design.
[a] Blood samples collected (pooled by litter) from fetus on GD 20 or cord/pups at birth from dose-range-finding or added extra animals for this purpose (see text for detail).
[b] Blood samples collected from culled pups on PND 4 may be pooled by litter and dose groups, if needed.
[c] Also weighed on the day attaining puberty (completion of preputial separation or vaginal patency).
[d] Collected from pups by sex and groups not assigned to any cohort (ideally 4/sex/dose group).
[e] From non-selected pups on PND 22: collect brain, spleen, thymus from 10 pups/sex/group or maximum possible, weigh and preserve. Also, preserve mammary and target tissues for microscopic analysis.
[f] Blood samples collected from pups on PND 14, 56, 76–89, 91 (see text for detail).
[g] All animals daily for balano-preputial separation or vaginal patency commencing before the expected day for achievement of these endpoints. Any abnormalities of genital organs, such as persistent vaginal thread, hypospadias or cleft penis, should be noted. Determine age and BW at balano-preputial separation or vaginal opening.

et al., 2011), the organization of the OECD 443 guideline is perceived to be difficult to follow. The criteria for selecting the highest dose is unclear (even though it recommends using toxicokinetic data generated in dose range-finding or other earlier studies). In addition, procedures for monitoring the systemic dose, for better interpretation and human relevance of the animal data, are not included. Saghir et al. (2013), on the other hand, offered criteria that can effectively guide dose selection and provide a direct example of the strategy for practical implementation of EOGRTS protocols. This paper examines ways to monitor systemic dose during the course of EOGRTS using core study animals and to select appropriate doses within dose-proportional range that are relevant to actual human exposure (Saghir, 2015; Saghir et al., 2012, 2013).

3. Role of kinetics in dose selection and incorporation into EOGRTS

Safety assessment of chemicals should focus on doses in animals that are relevant to human exposure while adequate to detect toxicity. One of the ways to determine the top dose for EOGRTS is to determine systemic dose proportionality and select the top dose based on the kinetically-derived maximum dose (KMD) at or slightly above the point of departure (POD) from dose proportionality (Marty et al., 2013; Saghir, 2015; Saghir et al., 2012, 2013). The POD from dose proportionality can be determined in a dose range-finding developmental study or in other repeat-dose toxicity studies as described by Saghir et al. (2012) and Saghir (2015). An effect observed in animals at the non-proportional systemic dose may not be relevant to the assessment of actual human risk; especially when the actual human exposure is many orders of magnitude lower than those used in animal studies. Additionally, it is recommended to have some kinetic information of chemicals in the test animal species along with likely human exposure estimates for appropriate margin of exposure before the initiation of reproductive toxicity studies with collection of further kinetic information in pregnant and lactating animals and in pups (ILSI/HESI, 2001; Cooper et al., 2006; Saghir et al., 2013). An example is given in Fig. 2 where the top dose for 2,4-D EOGRTS was selected based on KMD at slightly above the POD from proportionality of the systemic dose; the dose selected was half of the maximum tolerated dose and still several orders of magnitude higher than the expected human exposure (see Marty et al., 2013; Saghir et al., 2013 for detail). Determining systemic dose during the course of a reproductive/developmental study is also helpful in understanding the exposure at different life-stages (Fig. 3) for better interpretation of the human relevance of the results in test animals (Fegert et al., 2012; Marty et al., 2013; Saghir et al., 2013). In dietary exposure studies, the importance of adequately adjusting doses during different life-stages is emphasized in Fig. 3. Failure to adjust dietary concentrations can result in dramatically different systemic doses of test chemicals reflective of differences in bodyweight to food intake ratio, skewing the resulting risk assessment. In order to accomplish the determination of systemic dose at various developmental life stages, an approach for a single blood collection ($\leq$100 μl) at reproductive/developmental landmarks during the course of the study is proposed in Fig. 4 and Tables 1–4. Cord/pup along with maternal blood may be collected from animals used in the dose-range-finding (DRF) study or from dedicated groups in the main study as outlined in Fig. 4, pooled for each litter/dose group to achieve the minimum volume required for analysis. For the collection of blood from PND 4 pups, use of culled and extra animals in Cohort 3 of EOGRTS is recommended. Blood from each litter may be pooled when needed. The DRF study for EOGRT, when needed, can be designed to determine systemic dose in dams during gestation and in dams and pups at birth or even in dams and fetuses one day before parturition, if warranted, in a few designated animals in the DRF or main study. Similarly, milk can be obtained from

Table 3
Outline of EOGRTS for F1 cohort 1 with option for F2.

Cohort 1A: Reproductive systems and general toxicity assessment*	
Number	20/sex/group (1 male and 1 female per litter per group)
CO, PE, BW, FC	See table outlining general considerations for F1 animals
Vaginal smears	Daily after patency until cornified smear is recorded
Estrous cycles	Period of two weeks from around PND 75
Termination PND 91	
Vaginal smears	All: stage of estrous cycle for correlation with histopathology
Ovary	Follicle and corpora lutea counts
Blood	Hematology, Clinical Path, T4, TSH (10 randomly-selected animals)
Blood	***PND 91 for test chemical concentration (4 animals/sex/dose group)***
Urine	Urinalysis
Sperm analysis	Using one testis/epididymis (or vas deferens)
Tissues	Weights and histopathology
Immunotox (1 male or female per litter, all litters represented by at least 1 pup)	
Lymph nodes	Associated with and distant from the route of exposure
Spleen	½ for $CD4^+$ & $CD8^+$ T lymphocytes, B lymphocytes, NKC
Cohort 1B: Follow-up assessment of reproductive performance by mating F1 animals when needed	
Number	20/sex/group (1 male and 1 female per litter per group)
CO, PE, BW, FC	See table outlining general considerations for F1 animals
Vaginal smears	Pairing until evidence of mating
Cohabitation[a]	After PND 90 and before PND 120 avoiding siblings
F2 Pups	Sac on PND 4
Termination PND 98 or at birth of F2 pups[b]	
Vaginal smears	All: stage of estrous cycle for correlation with histopathology
Uteri[c]	All: presence and number of implantation sites
Blood	Hematology, Clinical Path, T4, TSH
Urine	Urinalysis
Sperm analysis	Using one testis/epididymis (or vas deferens)
Tissues	Weights and histopathology
Immunotox (1 male or female per litter, all litters represented by at least 1 pup)	
Lymph nodes	Associated with and distant from the route of exposure
Spleen	½ for $CD4^+$ & $CD8^+$ T lymphocytes, B lymphocytes, NKC

BW, body weight; CD4 and CD8, T cells; CO, clinical observation; FC, food consumption; NKC, natural killer T cells; PE, physical examination; PND, postnatal day; T4, thyroxin; TSH, thyroid stimulating hormones.
***PND 4 blood samples are collected from culled pups, few pups are used to collect PND 14 blood sample.**
Twenty-four hour or spot urine samples may be collected on days of blood collection to determine test chemical concentration.
Bold italic texts are proposed additions to improve the current EOGRTS design.
[a] Same as of P animals, only when generation of F2 animals are warranted.
[b] Samples will be processed however, analyzed only when the results from Cohort 1A are equivocal or suspected of reproductive or endocrine toxicity.
[c] The uteri of all F1 females, if applicable, are examined for the presence and number of implantation sites, in a manner which does not compromise histopathological evaluation.

dams during lactation from the DRF or designated main study animals (see OECD, 2012; Marty et al., 2013; Saghir et al., 2013 for detail). The collected blood (or processed plasma/serum) is analyzed for the parent chemical and/or metabolite(s) of interest.

4. Detailed and easy to follow steps for EOGRTS

An easily followed general outline of the EOGRTS, beginning before cohabitation through their final termination, is presented in Table 1. The outline also includes blood sampling (a single sample of $\leq$100 µl) at different life stages in order to determine changes in the potentially fluctuating systemic dose (Figs. 2 and 3), and for better understanding of the systemic exposure and associated human relevance of the outcome. Collection of one blood sample during designated life-stages is considered sufficient due to likely steady-state systemic dose with diurnal fluctuations based on the mode of dosing (e.g., dietary, through drinking water, daily oral gavage). When a single sample-based approach fails to adequately establish KMD of a chemical, collection of up to three blood samples at specific times needs to be considered as described by Saghir et al. (2006, 2013). Timing for the collection of blood sample(s) will depend on the kinetics of the test chemical; see Saghir (2015) and Saghir et al. (2006) for detail. The current EOGRTS design and suggested modifications to assess the systemic dose at different life stages are outlined in Figs. 1 and 4, respectively. Table 2 and Figs. 1 and 4 outline the EOGRTS processes for the F1 pups from their birth through maturation including blood sampling for the assessment of a systemic dose. Reproductive systems and general toxicity assessments for the Cohort 1A and reproductive performance, when triggered, of Cohort 1B pups along with the relationship with the systemic dose of the test chemicals and/or metabolite(s) are outlined in Table 3. The triggers to mate Cohort 1B animals to generate F2 animals is based on developmental landmarks (e.g., anogenital distance, nipple retention, puberty onset) in F1 animals. Table 4 outlines procedures for the neurotoxicity evaluation of test chemicals in Cohorts 2A and 2B and immunotoxicity evaluation in Cohort 3. A list of neurobehavioral assessments are presented in Table 5.

5. Conclusion

The EOGRT studies are complex and require a close and committed conversation among registrants, laboratories conducting EOGRTS, and regulatory agencies. The inclusion of additional studies for immunotoxicity assessment must also be carefully considered prior to finalization of the protocol; registrants must have a clear idea of the data needed to address the critical questions for each test chemical, and that the complex approach to a large multipurpose study is warranted for both the critical

Table 4
Outline of EOGRTS for F1 cohort 2 and 3.

Cohort 2A: neurobehavioral testing and neurohistopathology assessment as adults	
Number	10/sex/group (1 male or 1 female per litter per group)
CO, PE, BW, FC	See table outlining general considerations for F1 animals
Auditory startle test[a]	PND 24 ± 1
FOB[b]	Between PND 63 and PND 75
Motor activity[c]	Between PND 63 and PND 75
Termination after PND 75 and before PND 90	
Blood	***For test chemical concentration***
Tissues	Brain weight and full neurohistopathology - perfusion fixation
Brain (examination)[d]	Multiple section from different regions of the brain
Cohort 2B: neurohistopathology assessment at weaning (PND 21 or PND 22)	
Number	10/sex/group (1 male or 1 female per litter per group)
CO, PE, BW, FC	See table outlining general considerations for F1 animals
Termination PND 21 or PND 22	
Tissues	Brain weight and full neurohistopathology - perfusion fixation (optional)
Brain (examination)[d]	Multiple section from different regions of the brain
Blood	***For test chemical concentration***
Cohort 3: developmental immunotoxicity assessment	
Number	10/sex/group (1 male or 1 female per litter per group)
CO, PE, BW, FC	See table outlining general considerations for F1 animals
Termination PND 56 ± 3	
Blood	***For test chemical concentration***
Assays[e]	TDAR

BW, body weight; CO, clinical observation; FC, food consumption; FOB, functional observation battery; PE, physical examination; PND, postnatal day; TDAR, T-cell-dependent antibody response.
Twenty-four hour or spot urine samples may be collected on days of blood collection to determine test chemical concentration.
Bold italic texts are proposed additions to improve the current EOGRTS design.
[a] Each session consists of 50 trials (5 blocks of 10 trials).
[b] In home cage and to a standard arena for observation (open field), see table for details.
[c] By using automated activity recording apparatus capable of detecting increase and decrease in activity.
[d] Multiple sections from olfactory bulbs, cerebral cortex, hippocampus, basal ganglia, thalamus, hypothalamus, mid-brain (thecum, tegmentum, and cerebral peduncles), brain-stem and cerebellum. For Cohort 2A only, the eyes (retina and optic nerve) and samples of peripheral nerve, muscle and spinal cord are examined.
[e] T-cell dependent antibody response assay, i.e. serum IgM antibody titres (sensitization to Sheep Red Blood Cells [SRBC] or Keyhole Limpet Hemocyanin [KLH]), or splenic IgM specific plaque-forming cells (PFC) units (sensitization to SRBC). Responses typically peak four (PFC response) or five (ELISA) days after intravenous immunization. Additional pups may be required from control to act as positive controls.

Table 5
Outline of the FOB for EOGRTS.

Home Cage & open field	Manipulative	Physiologic
Posture		
Involuntary Clonic & Tonic	Ease of removal	Temperature
Palpebral Closure	Ease of handling	Body weight
Piloerection	Muscle Tone	Pupil response
Salivation	Approach Response	Pupil size
Lacrimation	Touch Response	
Vocalizations	Auditory Response	
Rearing	Tail Pinch Response	
Gait Abnormalities	Righting Response	
Arousal	Landing Foot Splay	
Stereotypy	Forelimb Grip Strength	
Bizarre Behavior	Hindlimb Grip Strength	
Stains		
Respiratory Abnormalities		

question and the need for the data. Although EOGRTS is a complex design needed significant resources, when conducted with the proposed evaluations, especially systemic dose determination across life-stage, has the potential to reduce the needs to conduct several additional separate developmental and reproductive toxicity studies. EOGRTS, when designed properly, may reduce the needs for studies such as OECD 414, 415, 416, 421, and 422 or those listed in OPPTS and ICH guidelines, ideally to only one additional study in a non-rodent (likely rabbit) species or to only those needing to determine fetal abnormalities that cannot be assessed in EOGRTS. The EOGRTS may also eliminate the need to separately conduct DNT and/or DIT studies. Therefore, in our opinion, EOGRTS with the proposed modifications, or a variant of it based on the properties of the test chemicals and issues at hand, will accomplish an overall reduction in the use of resources including the number of animals used in a series of studies conducted to assess the developmental and reproductive toxicity (and possible mode-of-action studies) of test chemicals by consolidating them into one large multipurpose study. It is agreed that the benefits of consolidating developmental and reproductive toxicity studies into one large multipurpose study must be evaluated carefully in relation to the questions needing answers.

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Transparency document

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